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Qualitative Gas Chromatographic Analysis by Means of Retention Volume Constants—Behavior of Isomers

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The technique of using retention volume constants derived from gas chromatographic behavior on two columns has been extended to include isomeric members of homologous series of alcohols, ketones, and esters. Data are provided which show that normal and isomeric members of homologous series within a functional group class have the same functional group retention volume constant.

A method for qualitative gas chromatography, utilizing retention volume constants derived from retention volume data on two columns employing different liquid phases, has been described in a previous paper (2). The method has been shown to be applicable to chromatograms obtained either isothermally or with programmed temperature and with either packed or capillary columns. The first paper established the criteria for identification of normal alkanes, alkyl benzenes, alcohols, aldehydes, ketones, ethers, esters, thiaalkanes and thiols. This paper describes the establishment of criteria for isomeric compounds with particular reference to homologous series of esters, alcohols and methyl ketones which were subsequently utilized in the identification of the components of a complex mixture isolated from a natural product (1).

EXPERIMENTAL

Apparatus and Procedure:

The retention volume data used to establish the behavior of isomers on packed columns were obtained by means of a simultaneous dual column gas chromatograph which has been described in detail in a previous paper (3). Isothermal data were obtained for columns operated at 125° C with a flow rate of 60 cc/minute. Programmed temperature data were obtained with the same apparatus using an F&M Scientific Company Model 240 Temperature Programmer to automatically program the columns from 0° to 100° C at a rate of 7.5° C per minute. Temperature programmed chromatograms were begun at 0° C since it has been found that lower starting temperatures improve linearity of the retention volume versus carbon number plots.

As in the earlier studies (2) the column pairs selected were Carbowax 4000 and OPN. The load weights were 20% on 60-80 mesh untreated Chromosorb W. The column dimensions were six feet by

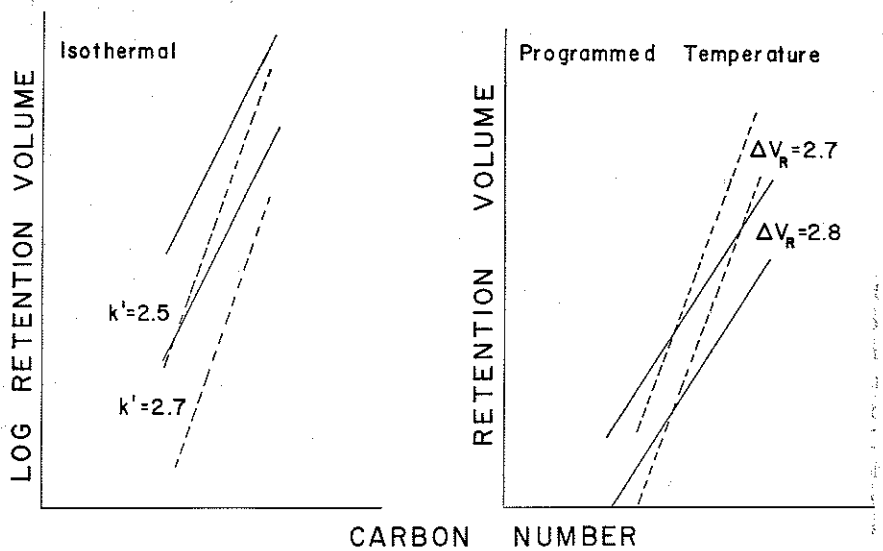

Figure 1. Retention behavior of isomeric alkyl acetates on OPN and CW-400 columns.
———— *n*-alkyl compounds, C_3-C_8
- - - - - - - - *iso*-alkyl compounds, C_5-C_8
"*iso*" = CH_3-CH(CH_3)-$(CH_2)_x$-R

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one-quarter inch outside diameter.

A set of isothermal data on Carbowax 4000 and Ucon polar 50HB-2000 columns was also obtained on a Research Specialties Company Series 600 Gas Chromotograph. These columns were twelve feet in length by one-quarter inch outside diameter and carried 5% load weights of the liquid phase on 80-100 mesh untreated Chromosorb W. The Carbowax column was operated at 65° C. and the "Ucon" polar column at 92° C. The constants derived from the retention data these columns provided were subsequently used to identify the volatile flavor and odor compounds isolated from banana fruit (1).

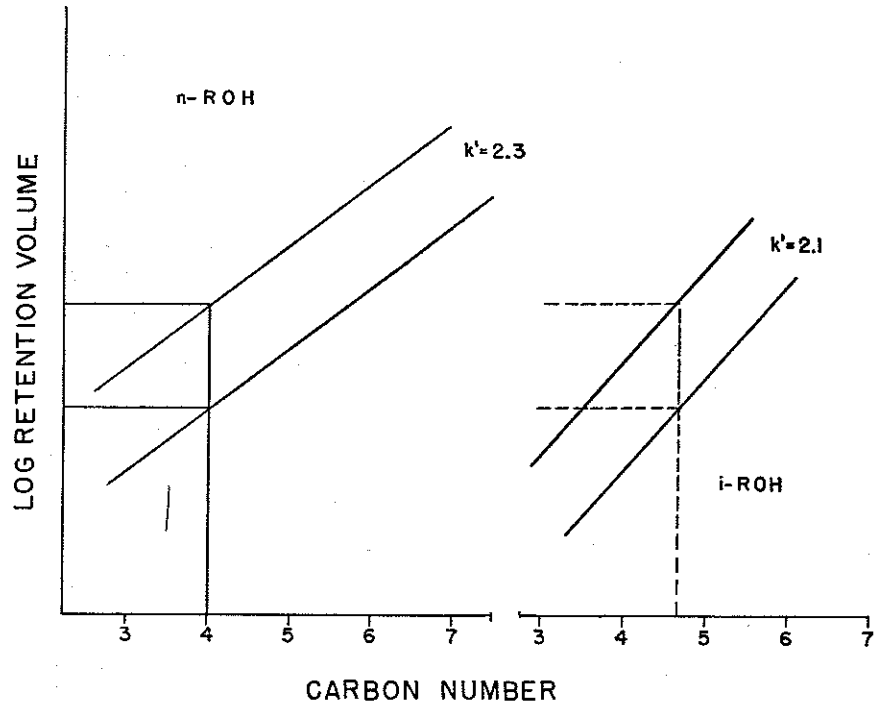

Figure 2. Graph showing distinction of normal from isomeric compounds having the same retention volume constant.

	M.W.	b.p.
Amyl propionate	144	165 °C
Butyl butyrate	144	166.4

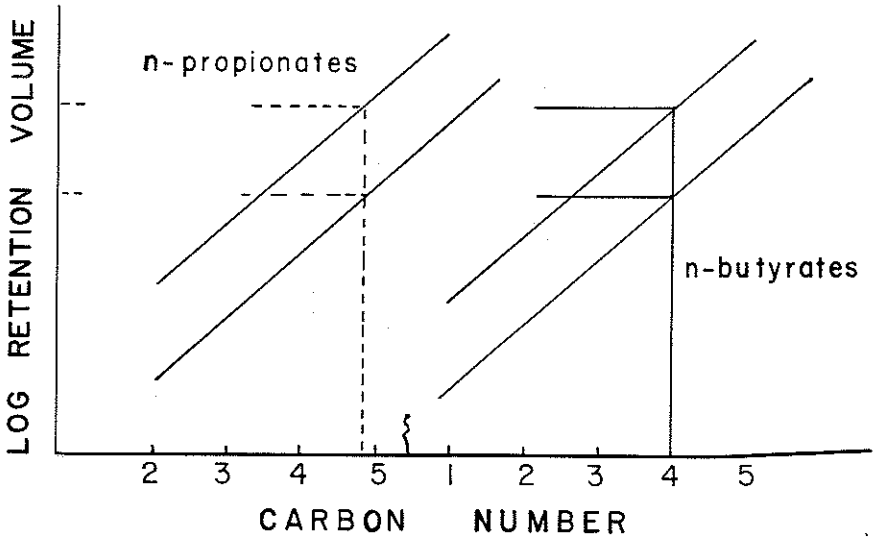

Figure 3. Graph showing identification of closely related isomers by means of retention volume constants.

Results and Discussion

The use of retention volume constants, both from isothermal and temperature programmed chromatograms, has been established for normal members of numerous homologous series of compounds (2). Since isomeric members of these series are frequently present, especially in heterofunctional mixtures isolated from natural products, the question arises whether such isomers may be distinguished. The situation may be illustrated by consideration of Figure 1 which shows that the isothermal log retention volume, and the programmed temperature retention volume plots versus carbon number show a parallel relationship for both the normal and isomeric series of alkyl acetates. It is seen that although the elution behavior of the two types may differ as shown by the change in slope of the parallel pairs, the retention volume constants are the same within the limits of precision of the measurements. This appears to be a general behavior as seen from the data in Table I. Accordingly, therefore, one may use the same retention volume constant to classify the functional group type for both normal and isomeric members.

The identification of the individual compounds within a functional group series is depicted in Figure 2 for the normal and iso alcohols. For example, the pair of retention volumes obtained on OPN and Carbowax 4000 would give a ratio of 2.2. The compound would therefore be classified as an alcohol by comparison with the tabulated values of retention volume constants. To identify the compound as an individual normal or iso alcohol, interpolation on the log retention volume versus carbon number plot will show that the pair of retention volumes would intersect the appropriate graphs at an integral carbon number only for the normal alcohol. They would not

give an integral number for the iso alcohol. In this case the compound is therefore normal butanol.

In connection with identification of the components of banana aroma the case of various esters has been studied in detail. The evaluation of the isothermal retention volume ratios ranging from acetates to valerates and including both the normal and iso compounds is tabulated in Table II. The values of the retention volume constants computed for 19 esters provided a mean which showed a precision that corresponds to the precision of one's ability to measure the retention volume (2). These data strongly support the argument that an ester, regardless of type, will provide a characteristic retention volume constant. Moreover, the same behavior is observed for programmed temperature operation. The data for acetates and butyrates (Table I) show that for a given temperature program these esters have a constant retention volume difference.

The identification of an individual compound within the functional group series is depicted by Figure 3 which illustrates the case of two esters of identical molecular weight having very close boiling points. A retention volume ratio of about 2.7 will identify the compound as an ester. Interpolation of the respective log retention-carbon number graphs would show that the compound must be butyl butyrate and could not be amyl propionate.

The results of these studies show the wide applicability of the retention volume constant technique and demonstrate that it is possible, therefore, in many cases to effect a positive identification of the components of a complex natural product mixture by use of retention volumes alone. The method is rapid, reproducible and reduces the need for expensive ancillary equipment.

Table I. Retention Volume Constants for Functional Group Isomers

Compound Type	Retention Volume Ratio (isothermal) CW/OPN	CW/UP	Retention Volume Difference OPN and CW-4000 (temp. prog.) 30-150° at 7° C/min.	0-150° at 5° C/min.
normal alcohols	2.3	4.2	1.9	.7
iso alcohols	2.1	4.1	1.7	.8
normal acetates	2.5	2.8	2.8	1.5
iso acetates	2.7	2.7	2.7	1.5
methyl ketones (normal)	3.0		4.3	1.9
methyl ketones (iso)	3.2		4.0	1.9
normal butyrates	2.6	2.7	2.7	1.5
iso butyrates	2.7	2.6	2.7	1.5

iso = CH_3-CH(CH_3)-$(CH_2)_x$-R R = functional group

Table II. Evaluation of Isothermal Retention Volume Constants of Esters

Carbowax 4000 and "Ucon" polar 50 HB-2000 Columns

Acetates	k	Propionates	k	Butyrates	k
n-butyl	2.9	ethyl	2.8	methyl	2.7
n-amyl	2.8	*n*-propyl	2.6	ethyl	2.6
n-hexyl	2.8	*n*-butyl	2.6	*n*-propyl	2.5
		n-amyl	2.6	*n*-butyl	2.5
				n-amyl	2.5
i-propyl	2.7			*i*-propyl	2.6
i-butyl	2.7			*i*-butyl	2.6
i-amyl	2.7			*i*-amyl	2.6
		Valerates	k		
		ethyl	2.6		

$k = V_R(CW)/V_R(UP)$ mean = 2.7 ± 0.2

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Literature Cited

1. McCarthy, A. I., Wyman, H. and Palmer, J. K., J. Gas Chromatog. **2**, No. 4: 121 (April 1964).
2. Merritt, C., Jr., Walsh, J. T., Anal. Chem. **34**, 903 (1962).
3. Merritt, C., Jr., Walsh, J. T., **ibid., 34,** 908 (1962).

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